

Art 34

10 Rec 07 NOV 2001

A COMPOSITION CONTAINING CARVACROL AND THYMOL FOR USE AS A
BACTERICIDE

5 The present invention relates to a composition
containing the natural substances carvacrol and thymol. The
composition exhibits a synergistic bactericidal effect
against Treponema, which causes severe muco-hemorrhagic
diseases, such as swine dysentery and severe diseases
affecting the hoofs of hoofed animals, particularly cloven-
10 hoofed animals, such as ruminants. The composition is
suitably administered to an animal via a diet composition,
drinking water or a drench bath.

20 Diseases caused by Treponema are common in animal
stocks. For example, an anaerobic spirochete, Treponema
hyodysenteriae, is considered to be the primary etiologic
agent of swine dysentery (SD). See for instance D.L. Harris
and R.J. Lyons (1992), Swine dysentery, in: Diseases of
swine, 7th edition, Iowa State University Press, Ames, Iowa,
USA. SD is a severe muco-hemorrhagic diarrhoea. Pigs
25 actually affected with SD usually consume very low amounts
of feed causing an essential reduction in growth and a
considerable economical loss. In Top Agrar (1998), Vol. 4,
page 52 it is reported that in Germany one third of the
piglets herds and as much as one half of the growing pig
30 herds probably are Treponema-positive. Large efforts are
therefore justified to prevent the diseases to be spread. It
is also well known that Treponema causes severe diseases
affecting the hoofs of hoofed animals, particularly cloven-
hoofed animals, such as ruminants.

35 M. Tiziana Baratta et al.: "Chemical
composition, antimicrobial and antioxidative activity
of laurel, sage, rosemary, oregano and coriander
essential oils" Journal of Essential Oil Research.,
vol. 10, no 6, 1998, pages 618-627, XP000929938 XX, XX
ISSN: 1041-2905, disclose the chemical composition of
five essential oils, derived from sage, rosemary,
oregano, laurel and coriander, and also the anti-

bacterial activity of these essential oils against a number of microorganisms.

WO 96/37210 describes a pharmaceutical composition wherein the active agent is an extract from certain plants, especially *Origanum vulgare*, *Thymus vulgaris* and *Mentha piperita*. In general, the extracted oil contains 3% thymol and 60-70% carvacrol

Furthermore, in WO 97/01348 it is disclosed a pharmaceutical composition comprising a herbal essential oil containing thymol and carvacrol as its main ingredients and pharmaceutically acceptable carrier. The total amount of thymol and carvacrol in said essential oil is at least 55%, preferably 70% by weight of said essential oil, and the ratio of carvacrol to thymol is at least 10. This pharmaceutical composition can be used in the prevention and treatment of coccidiosis in poultry.

A wide spectrum of antibiotics, such as streptomycin, bacitracin, neomycin, tylosin, gentamycin, chlortetracycline, virginamycin and lincomycin, have been reported to be effective in the treatment of SD. Where the disease is endemic, preventive medication is often added to the animal feed. However, it seems that the antibiotics become less and less effective and Top Agrar (1998), Vol. 4, page 52, reports a resistance ratio of Treponema of 99% to tylosin, 92% to lincosamycin and 48% to tiamulin in 1997.

However, in recent years there has been an intense debate about the use of chemical and antibiotic growth promoters and in many countries a ban on this type of feed additives is being considered. Thus, there is an urgent need for agriculture to develop substances which are in line with reliable and generally accepted practice and not of a medicinal nature.

One objective of the present invention is to provide natural substances as active agents, which are suitable for the administration to the animal via a diet, drinking water or a drench bath for the cure, prevention or alleviation of the diseases caused by Treponema. Another objective is to reduce the negative effects on the growth.

According to the invention it has been found that a composition, containing the natural substances carvacrol in an amount of 5 ppm to 90% by dry weight and thymol in an amount of 5 ppm to 80% by dry weight, in a weight ratio between 1:5 and 10:1, preferably between 2:3 and 4:1, exhibits a synergistic effect against Treponema and thereby diminishing the negative effect these bacteriae have on the health and growth of animals. By the expression "a natural substance" is in this context understood a substance which consists of compounds occurring in nature and that is obtained from natural products or through synthesis. The composition is usually administered as a diet composition or as a drinking water containing carvacrol and thymol in amounts of 5-2000 ppm, preferably 20-600 ppm, calculated on the dry weight of the diet composition including nutritive

substances or on the weight of the drinking water. The composition may also be present in an amount of 0.2-30% by weight in a drench bath for the treatment of the hoofs of hoofed animals, particularly cloven-hoofed animals, such as ruminants. The invention also includes a premix and a diet additive that may be used in the preparation of the diet composition as well as a drinking water supplement and a drench bath supplement.

The composition and the diet composition may also contain other natural substances which enhance the health and improve the growth. In the diet composition these substances are normally present in an amount of 0.1 to 30 ppm, calculated on the dry weight. Examples of such substances and their amounts are 1-5 ppm guaiacol, 1-5 ppm eugenol, 0.1-2 ppm capsasciin and 1-20 mg tannin. Other suitable ingredients in the diet composition are flavourings of natural substances. They are usually present in an amount of 0.2-50 ppm, calculated on the dry weight of the diet composition. Examples of suitable flavourings and their amounts are 0.05-0.5 ppm creosol, 0.1-5 mg anethole, 0.1-2 ppm of deca-, undeca- and/or dodecalactones, 0.1-2 quinoleine, 0.1-2 ppm ionones and/or irone, 0.05-1 ppm gingerol, 0.05-2 ppm piperine, 0.05-1 ppm propylidene and/or butylidene phtalides and 0.1-5 ppm amyl and/or benzyl salicylate.

The incorporation of active ingredients into the diet composition is usually carried out by preparing a premix of the active compounds carvacrol and thymol and other suitable additives. Such a premix may contain 1-10% by dry weight of carvacrol and thymol, 0-40% by dry weight of growth improving additives, flavourings and health enhancing additives, and 50-99% by weight of an absorbing support. The support may contain, for example, 40-50% by weight of wood fibres, 8-10% by weight of stearin, 4-5% by weight of curcuma powder, 4-5% by weight of rosemary powder, 22-28% by weight of limestone, 1-3% by weight of a gum, such as gum arabic, 5-50% by weight of sugar and/or starch and 5-15% by weight of water.

This premix can then be mixed with common feed components, such as vitamins, enzymes, mineral salts, ground cereals, protein-containing components, carbohydrate-containing components, wheat middlings and/or brans in the preparation of a diet composition additive which contains 0.2-5% by weight of the premix. The diet composition additive is then finally added to the diet composition in such quantities that the feed will contain 5-2000 ppm, preferably 20-600 ppm, of the active mixture. The diet composition additive normally constitutes 0.3-3.5% by weight of diet composition.

The diet composition according to the invention usually contains, calculated on the dry weight of the feed, the following ingredients:

- a) 0-80%, preferably 10-70%, by weight of cereals,
 - b) 0-30%, preferably 1-12%, by weight of fat,
 - c) 0-85%, preferably 10-50%, by weight of protein containing nutritious substances of a type other than cereals, and
 - d) 1-500 ppm, preferably 10-100 ppm, of the mixture.
- The total amounts of a)-d) are preferably at least 80% by weight.

When preparing the diet composition, the diet composition additive can be mixed with the dry ingredients consisting of cereals, such as ground or crushed wheat, oats, barley, maize and rice; vegetable protein feed based on e.g. rapeseed, soya bean and sunflower; animal protein feed, such as blood meal, meat and bone meal and fish meal; molasses; and milk products, such as various milk powders and whey powders. After mixing all the dry additives, the liquid ingredients and ingredients, which after heating become liquid, can be added. The liquid ingredients may consist of lipids, such as fat, for example slaughter fat and vegetable fat, optionally liquefied by heating, and/or of carboxylic acids, such as a fatty acid. After thorough mixing, a mealy or particulate consistency is obtained, depending on the degree of grinding of the ingredients. To prevent separation during storage, water should preferably

be added to the animal feed, which then is subjected to a conventional pelletising, expanding or extruding process. Any excess water can be removed by drying. If desired, the resulting granular animal feed can also be crushed to a smaller particle size.

The drinking water supplement may contain 2-90% by dry weight, preferably 10-50% by dry weight, of carvacrol and thymol. Beside carvacrol and thymol the supplement also contains 10-98% by dry weight of a large number of other ingredients. Common ingredients are mineral salts, vitamins, natural substances enhancing the health and growth, flavourings, water-soluble or water-dispersable carriers, such as sugars, powdered milk, milk-by-products and cellulose derivatives, dispersing agents and stabilisers, such as water-soluble or water-dispersable polymers. Suitable examples of natural substances enhancing the health and growth have earlier been described. When preparing the drinking water, the supplement is normally added to the water in such an amount that the concentration of the natural substance becomes 5-2000 ppm, preferably 20-600 ppm.

The drench bath supplement may contain 30-98% by dry weight of carvacrol and thymol and 2-70% by weight of mineral salts, water-soluble or water-dispersable carriers, dispersing agents and/or stabilisers, such as water-soluble or water-dispersable polymers.

Within the scope of the invention, it is also possible to produce a suspension of the diet composition. This is especially convenient if the feed is prepared for immediate consumption.

The present invention will now be further illustrated by the following Examples.

Example 1

The antimicrobial activity of the composition of the invention towards *Treponema innocens* and *Treponema hyodysenteriae* was determined in vitro. In the tests the following organisms, growth media, culture conditions and evaluation method were used.

Organisms: Treponema innocens, ATTC 29796
 Treponema hyodysenteriae, ATCC 31212

5 Growth media: Caseine-peptone soymeal-peptone agar USP
 (Caso-Agar, Merch No. 5458) + 5% Sheep
 blood

10 Culture conditions: Anaerobic incubation at 37°C for 4-6
 days

Evaluation method: Agar dilution test (according to DIN
 58940, teil 6)

15 Agar plates were prepared by using the growth media,
to which 10% by weight of a solution of carvacrol and/or
thymol in polypropylene glycol had been added.

20 Cell suspensions with a concentration of 10.9 cfu/ml
were prepared of each of the organisms. The single
suspensions were then distributed on the agar surface using
a Multipoint inoculator applying 1µl to a final surface of
about 0.5 cm². For every concentration of carvacrol and/or
thymol two parallel plates were inoculated and on each plate
three inoculation points of each of the two organisms are
applied. After the inoculation period the growth of the
25 organisms were observed. If no growth was observed, the
concentration of carvacrol and/or thymol was in the next
test reduced to half. The minimum concentration of carvacrol
and/or thymol leading to a total suppression of bacterial
growth is noted as the MIC value (minimal inhibitory
30 concentration) of the active components or compounds. The
following results were obtained.

Table 1. Minimal inhibitory concentration

Test No.	Active comp und	MIC value, ppm	
		Treponema innocens	Treponema hyodysenteriae
1	Thymol	625	625
2	Carvacrol	313	313
3	2/3 Thymol 1/3 Carvacrol	156	156
4	1/2 Thymol 1/2 Carvacrol	156	156
5	1/3 Thymol 2/3 Carvacrol	< 78	< 78

From the results it is evident that the composition of the invention exhibits a synergistic antimicrobial effect on the tested organisms.

Example 2

In order to determine the efficacy of the mixture of the invention to reduce the occurrence of *Treponema* some tests were performed with grower pigs (25 kg to 100 kg), which were put on commercial diets with the following composition.

Crude protein, %	24.0
Crude fat, %	6.0
Crude fiber, %	3.5
Crude ash, %	5.0
Lysine, %	1.35
MJDE, kg	14.3

This diet was formulated by mixing suitable amounts of wheat, lupin kernel, canola meal, rice pollard, meat meal, blood meal and tallow. To the diet administered to the experimental group 100 ppm of a mixture containing 67 ppm carvacrol and 33 ppm thymol were added.

During the growing phase animal faeces were controlled for presence of Treponema and development of dysentery was recorded. The following results were obtained.

5 **Table 2. Occurrence of swine dysentery and Treponema in faeces**

	No. of animals controlled	Presence of Treponema in faeces	Development of swine dysentery
Control	23	8	1
Experimental	10	0	0

10 These results clearly indicate that the occurrence of Treponema is much lower when the mixture of the invention is added to the feed in comparison to a negative control.

Example 3

15 The purpose of the following experiments were to investigate the effect of the mixture according to the present invention to increase animal growth and inhibition of the growth of Treponema. Forty male pigs in two pens were allocated to each treatment. The pigs were weighed individually at an age of 46 days, when the experimental diets were introduced. The experiment continued for 42 days.

20 All the diets in the tests contained the following basic composition.

Crude protein, %	24.4
Crude fat, %	6.3
Crude fiber, %	3.5
Crude ash, %	5.3
Lysine, %	1.35
MJDE, kg	14.5

25 This composition was formulated by mixing suitable amounts of wheat, lupin kernel, canola meal, rice pollard, meat meal, blood meal and tallow.

In a control test A 100 ppm Olaquinox and 25 ppm Tiamulin had been added to the basic composition while in tests I and II according to the invention the diets contained 100 ppm and 200 ppm of the mixture of carvacrol and thymol disclosed in Example 2. In a control test B the diet consisted of the basic composition. The following results were obtained.

Table 3. Effect on the performance of pigs between 46 and 67 days of age.

Tests	A	B	I	II
Start weight (kg)	15.3	15.2	15.3	15.2
Final weight (kg)	28.6	27.0	26.9	27.2
Daily gain (g)	666	588	578	595
Feed intake (g/d)	1100	1000	933	971
Feed gain	1.65	1.70	1.61	1.63

Table 4. Effects on the performance of pigs during 67 to 88 days of age.

Tests	A	B	I	II
Final weight (kg)	42.8	41.9	40.8	41.2
Daily gain (g)	665	695	655	671
Feed intake (kg/d)	1.38	1.42	1.32	1.37
Feed gain	2.04	2.05	2.02	2.05

From these results one can clearly see that the addition of the mixture of the invention increases the growth especially in the period between 46 and 67 days of age.

The effect of diets containing the active mixture of the invention and the presence of *Treponema* in faeces was investigated by feeding four groups with five pigs in each group on the diets used in control test A and B and in tests I and II. The faeces of each pig was examined in regard to the presence of *Treponema* after the pigs have been fed on the diets 4, 8 and 16 days. The following results were obtained.

Table 5. Occurrence of Treponema in faeces

Time, days	Samples with Treponema			
	Control A	Control B	Test I	Test II
4	1	3	4	4
8	3	3	0	0
16	0	0	0	0

5 From the results it is evident that the pigs fed on the diet according to the invention did not have Treponema in their faeces after 8 days while the pigs fed on the diets in Controls A and B needed treatment for 16 days.